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RealStar®
Zika Virus RT-PCR Kit U.S.
Instructions for Use

For Use Under the Emergency Use Authorization (EUA) Only

Version 1.2 05/2016

RealStar®
Zika Virus
RT-PCR Kit U.S.

For use with

ABI Prism® 7500 SDS (Applied Biosystems)
ABI Prism® 7500 Fast SDS (Applied Biosystems)
LightCycler® 480 Instrument II (Roche)
Rotor-Gene® 6000 (Corbett Research)
Rotor-Gene® Q 5/6 plex/MDx Platform (QIAGEN)
CFX96™ Real-Time PCR Detection System (BIO-RAD)
CFX96™ Deep Well Real-Time PCR Detection System (BIO-RAD)

For Use Under the Emergency Use Authorization (EUA) Only

IVD — For *in vitro* diagnostic use

℞ only — For prescription use only

EUA — For use only under Emergency Use Authorization

REF — Product No.: 591023

Σ — 96 rxns

Store at -25°C ... -15°C

May 2016

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MAN-591020-EN-01

Content

1. Intended Use

The RealStar® Zika Virus RT-PCR Kit U.S. is a real-time RT-PCR test intended for the qualitative detection of RNA from the Zika virus in serum or urine (collected alongside a patient-matched serum specimen) from individuals meeting CDC Zika virus clinical criteria (e.g., clinical signs and symptoms associated with Zika virus infection) and/or CDC Zika virus epidemiological criteria (e.g., history of residence in or travel to a geographic region with active Zika virus transmission at the time of travel, or other epidemiologic criteria for which Zika virus testing may be indicated), by laboratories in the United States that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests, or by similarly qualified non-U.S. laboratories.

Results are for the identification of Zika virus RNA. Zika virus RNA is generally detectable in serum during the acute phase of infection (approximately 7 days following onset of symptoms, if present). Positive results are indicative of current infection. Laboratories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude Zika virus infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The RealStar® Zika Virus RT-PCR Kit U.S. is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

2. Kit Components

Lid Color	Blue	Purple	Green	Red	White
Component	Master A	Master B	Internal Control	Positive Control	PCR grade Water
Number of Vials	8	8	1	1	1
Volume [µl/Vial]	60	180	1000	250	500

3. Storage

- The RealStar® Zika Virus RT-PCR Kit U.S. is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact altona Diagnostics GmbH for assistance.
- All components should be stored between -25°C and -15°C upon arrival.
- Always check the expiration date and do not use reagents beyond the expiration date.
- Repeated thawing and freezing of Master reagents (more than twice) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently.
- Storage between +2°C and +8°C should not exceed a period of two hours.
- Protect Master A and Master B from light.

4. Material and Devices required but not provided

- Appropriate real-time PCR instrument:
  - ABI Prism® 7500 SDS (Applied Biosystems, Cat. No. 4351104)
  - ABI Prism® 7500 Fast SDS (Applied Biosystems, Cat. No. 4351106)
  - LightCycler® 480 Instrument II (Roche, Cat. No. 05015278001)
  - CFX96™ Real-Time PCR Detection System (BIO-RAD, Cat. No. 185-5195)
  - CFX96 ™ Deep Well Real-Time PCR Detection System (BIO-RAD, Cat. No. 185-4095)
  - Rotor-Gene® 6000 (Corbett Research, Cat. No. P/N 6500, P/N 65H0, P/N 6600)
  - Rotor-Gene® Q 5/6 plex/MDx Platform (QIAGEN, Cat. No. 9001570, 9001590, 9002035)
- Appropriate nucleic acid extraction kit:
  - QIAamp® Viral RNA Mini Kit (QIAGEN, Cat. No. 52906 or 52904)
- Desktop centrifuge with a rotor for 2 mL reaction tubes (Eppendorf 5415C or equivalent)
- Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates
- Vortex mixer (VWR 58810-163 or equivalent)
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)
- Nuclease-Free Water (not DEPC-Treated), Life Technologies (Cat. No. 4387936) or equivalent

NOTE

> ⚠ ***Please ensure that instruments have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.***

5. Background Information

Zika virus is an enveloped, single stranded (+) RNA virus of the family *Flaviviridae*. Like many other members of the family it is mainly transmitted by mosquitoes of the genus *Aedes*. In 1947, the virus was isolated from a rhesus monkey in Uganda for the first time. The first human case of Zika virus infection was discovered in 1968 in Nigeria. Initially, evidence for Zika virus infections has only been found in patients from Africa and South-East Asia. In 2007 the virus caused a large outbreak in Micronesia and other islands in the Pacific Ocean. French Polynesia, Easter Islands and Cook Islands were affected in 2013. Since 2015 the virus is also endemic to South America, where since then large numbers of suspected cases were recorded. Fever, rash and arthralgia are common symptoms and signs of Zika virus infections which nevertheless usually are mild and self-limiting.

Because Zika, Dengue and Chikungunya virus are endemic to the same geographical regions and cause similar symptoms, definite identification of the etiological agent is only possible with laboratory testing. Zika virus detection should be done early after symptom onset when using RT-PCR (<7 days for serum, <14 days for urine). Serum should be tested with a serological assay if the specimen was collected more than 7 days after symptom onset. Antibody assays commonly cross-react with closely related flaviviruses and detection of neutralising antibodies by plaque reduction assays is laborious and can only be done in specialised laboratories.

NOTE

> ⚠ ***Due to the molecular evolution of RNA viruses, there is an inherent risk for any PCR based test system that accumulation of mutations over time may lead to false negative results.***

6. Product Description

1. The RealStar® Zika Virus RT-PCR Kit U.S. is a real-time RT-PCR test intended for the qualitative detection of RNA from the Zika virus in serum or urine (collected alongside a patient-matched serum specimen) from individuals meeting CDC Zika virus clinical criteria (e.g., clinical signs and symptoms associated with Zika virus infection) and/or CDC Zika virus epidemiological criteria (e.g., history of residence in or travel to a geographic region with active Zika transmission at the time of travel, or other epidemiologic criteria for which Zika virus testing may be indicated). The reagent system includes a heterologous amplification system (Internal Control) to identify possible RT-PCR inhibition and to confirm the integrity of the reagents of the kit.

   The test is based on real-time RT-PCR technology, utilizing reverse transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA), polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labelled with fluorescent reporter and quencher dyes.

   Probes specific for Zika virus RNA are labeled with the fluorophore FAM™. The probe specific for the target of the Internal Control (IC) is labeled with the fluorophore JOE™. Using probes linked to distinguishable dyes enables the parallel detection of Zika virus specific RNA and the Internal Control in the corresponding detector channels of the real-time PCR instrument.

2. The workflow starts with taking whole blood or urine from the patient. To generate serum from the collected whole blood the specimen is incubated at room temperature to allow clotting. Afterwards the clot is removed by centrifugation and the resulting supernatant (the serum) transferred into a separate tube. For nucleic acid extraction the QIAamp® Viral RNA Mini Kit (QIAGEN, Hilden, Germany) must be used. 140 µl of serum or urine have to be mixed with 560 µl of AVL. The extraction of the RNA is performed following the manufacturer's instructions for the QIAamp® Viral RNA Mini Kit (QIAGEN). The extracted RNA will afterwards serve as template for analysis with the RealStar® Zika Virus RT-PCR Kit U.S.

   The temperature cycling and signal detection can be done with the real-time PCR instruments listed as followed:

   - ABI Prism® 7500 SDS (Applied Biosystems)
   - ABI Prism® 7500 Fast SDS (Applied Biosystems)
   - LightCycler® 480 Instrument II (Roche)
   - CFX96™ Real-Time PCR Detection System (BIO-RAD)
   - CFX96™ Deep Well Real-Time PCR Detection System (BIO-RAD)
   - Rotor-Gene® 6000 (Corbett Research)
   - Rotor-Gene® Q 5/6 plex/MDx Platform (QIAGEN)

   The evaluation of the results and positive or negative calling of the samples is the last step of the workflow.

3. The RealStar® Zika Virus RT-PCR Kit U.S. contains of:

- Two Master reagents (Master A and Master B)
- Template Internal Control (IC)
- Positive Control
- PCR grade water

Master A and Master B contain all components (buffer, enzymes, primers, and probes) to allow reverse transcription, PCR mediated amplification and target detection (Zika virus specific RNA and Internal Control) in one reaction setup.

The following control materials are provided and to be used with the RealStar® Zika Virus RT-PCR Kit U.S.:

a) Internal Control (IC)
The Internal Control contains a defined copy number of an “artificial” RNA molecule with no homologies to any other known sequences. It has to be added to the nucleic acid extraction procedure and is reverse transcribed, amplified and detected in parallel to the Zika virus specific RNA. The function of the Internal Control is to ensure the integrity of Zika virus specific real-time RT-PCR results by indicating potential RT-PCR inhibition.

b) PCR grade water
The PCR grade water is to be used as negative control for the RT-PCR reaction. Its function is to indicate contamination of RT-PCR reagents.

c) Positive Control Zika virus
The Positive Control consists of an *in vitro* transcript which contains the target sequence used by the RealStar® Zika Virus RT-PCR Kit U.S. for the detection of Zika virus specific RNA. The Positive Control is used as positive control for the RT-PCR and verifies the functionality of the Zika virus RNA specific RT-PCR detection system, which is included in the RealStar® Zika Virus RT-PCR Kit U.S..

d) Negative Process Control (NPC)
Apart from the controls provided with the RealStar® Zika Virus RT-PCR Kit U.S. a water sample (Nuclease-Free Water (not DEPC-Treated), Life Technologies (Cat. No. 4387936) or equivalent) should be included in each run as Negative Process Control (NPC) to monitor the nucleic acid extraction procedure.

7. Test Principle

The test consists of three processes in a single tube assay:

- Reverse transcription of target RNA and Internal Control RNA to cDNA
- PCR amplification of target and Internal Control cDNA
- Simultaneous detection of PCR amplicons by fluorescent dye labelled probes

The RealStar® Zika Virus RT-PCR Kit U.S. is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The Zika virus specific primer and probe set is designed to detect RNA from the Zika virus in serum and urine from patients presenting signs and symptoms of the Zika virus infection in conjunction with epidemiological risk factors.

One-step RT-PCR assays are one-tube assays that first reverse-transcribe specific RNA templates into cDNA copies. This cDNA then undergoes a polymerase chain reaction (PCR) that utilizes a thermocyclic heating and cooling of the reaction to logarithmically amplify a specific region of DNA. The probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5’ nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle.

8. Limitations and Precautions

- Negative results do not preclude infection with Zika virus and should not be used as the sole basis of a patient treatment/management decision. All results should be interpreted by a trained professional in conjunction with review of the patient's history and clinical signs and symptoms.
- Interpretation of RT-PCR test results must account for the possibility of false negative and false positive results. False negative results can arise from:
  - poor sample collection or
  - degradation of the viral RNA during shipping or storage or
  - specimen collection conducted prior to symptom onset
  - specimen collection after nucleic acid can no longer be found in the patient (approximately 7 days post-onset of symptoms for sera)
  - failure to follow the authorized assay procedures
  - failure to use authorized extraction kit and platform
- Performance has only been established with the specimen types listed in the Intended Use. Other specimen types have not been evaluated and should not be used with this assay.
- Good laboratory practice is essential for proper performance of this assay. Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded. False positive results may occur from cross-contamination by target organisms, their nucleic acids or amplified product.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test. Improper collection, storage, or transport of specimens may lead to false negative results.
- The impact of the administration of Zika virus vaccines and/or therapeutics on the ability to detect Zika Virus RNA in patient specimens has not been evaluated.
- This assay must not be used on the specimen directly. Appropriate nucleic acid extraction using the QIAamp® Viral RNA Mini Kit must be conducted prior to using this assay.

- The presence of RT-PCR inhibitors may cause false negative or invalid results.
- Potential mutations within the target regions of the virus genome covered by the primer and/or probes of the test may result in failure to detect the presence of the pathogen.

9. Warnings and Precautions

- This assay is for *in vitro* diagnostic use under the FDA Emergency Use Authorization only.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- Use of this product is limited to specified laboratories and clinical laboratory personnel who have been trained on authorized instruments.
- Laboratories are required to report all positive results to the appropriate public health authorities.
- Results need to be interpreted in conjunction with clinical signs, symptoms and travel history of the patient or contact information.
- Do not use reagents from other manufacturers with this assay.
- Specimens should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Follow necessary precautions when handling specimens. Use personal protective equipment (PPE) consistent with current guidelines. Additional information on safe handling of Zika virus specimens can be found at: http://ww.cdc.gov/zika/state-labs/index.html
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimen and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.

- Use separated and segregated working areas for (i) specimen preparation, (ii) reaction set-up and (iii) amplification/detection activities. Workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering different areas.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Do not use components of the kit that have passed their expiration date.
- Discard sample and assay waste according to your local safety regulations.
- Performance of the RealStar® Zika Virus RT-PCR Kit U.S. has only been evaluated for serum and urine specimens in conjunction with the QIAamp® Viral RNA Mini Kit.
- Due to the relatively fast molecular evolution of RNA viruses, there is an inherent risk for any RT-PCR based test system that accumulation of mutations over time may lead to false negative results.

NOTE

⚠ ***Any positive specimen result (urine or serum) is considered a positive diagnosis of Zika virus infection. Zika virus specific RNA detected results must be reported to the appropriate Public Health agency. Please refer to the CDC website for the most update information on patient follow up. http://www.cdc.gov/zika/hc-providers/clinical-guidance.html.***

10. Instructions for Use

10.1 RNA Extraction using the QIAamp® Viral RNA Mini Kit

Details on the RNA extraction process using the QIAamp® Viral RNA Mini Kit (QIAGEN) are given below. Carefully read the manufacturer's instructions for use (QIAamp® Viral RNA Mini Handbook 04/2010) for general handling instructions.

10.1.1 Addition of carrier RNA and Internal Control template to Buffer AVL

Check Buffer AVL for precipitate, and if necessary incubate at 80°C until precipitate is dissolved. Prepare the required amount of Buffer AVL freshly. Calculate the volume of "Buffer AVL/carrier RNA/Internal Control template"-mix needed per batch of samples by selecting the number of samples to be simultaneously processed from Table 1. Mix the reagents by inverting the tube 10 times. The number of samples is defined by the number of patient samples to be tested plus one additional Negative Process Control (NPC; Nuclease-Free Water (not DEPC-Treated), Life Technologies (Cat. No. 4387936) or equivalent).

Table 1: Volumes of Buffer AVL, carrier RNA and Internal Control template required for the QIAMP® Viral RNA Mini Kit procedure

No. of Samples	Vol. of Buffer AVL[a] (mL)	Vol. of carrier RNA[a] (µl)	Vol. of IC template (green lid)[b] (µl)
1	0.56	5.60	6.00
2	1.12	11.20	12.00
3	1.68	16.80	18.00
4	2.24	22.40	24.00
5	2.80	28.00	30.00
6	3.36	33.60	36.00
7	3.92	39.20	42.00
8	4.48	44.80	48.00
9	5.04	50.40	54.00
10	5.60	56.00	60.00
11	6.16	61.60	66.00
12	6.72	67.20	72.00
13	7.28	72.80	78.00
14	7.84	78.40	84.00
15	8.40	84.00	90.00
16	8.96	89.60	96.00
17	9.52	95.20	102.00
18	10.08	100.80	108.00
19	10.64	106.40	114.00
20	11.20	112.00	120.00
21	11.76	117.60	126.00
22	12.32	123.20	132.00
23	12.88	128.80	138.00
24	13.44	134.40	144.00

[a] supplied with the QIAamp® Viral RNA Mini Kit

[b] supplied with the RealStar® Zika Virus RT-PCR Kit U.S.

Do not forget to reconstitute buffers AW1 and AW2 with 96-100% ethanol (see manufacturer guidelines for more information).

10.1.2 Extraction process

1. Pipette 560 µl of prepared Buffer AVL containing carrier RNA and Internal Control template into a 2 mL labelled microcentrifuge tube.
2. Add 140 µl of specimen or 140 µl of water for Negative Process Control (NPC) to be extracted to the 2 mL labelled RNase-free microcentrifuge tube and mix by pulse-vortexing for 15 seconds.
3. Incubate specimen(s) and control at room temperature (15–25°C) for 10 minutes.
4. Briefly centrifuge the tubes to remove drops from the inside of the lid.
5. Add 560 µl of 96-100% ethanol to each specimen and control tube, and mix by pulse-vortexing for 15 seconds. After mixing, briefly centrifuge the tubes to remove drops from inside the lid.
6. For each specimen and control, place a QIAamp® spin column into a 2 mL collection tube (from the QIAamp® Viral RNA Mini Kit). Be sure to label the top of the columns clearly.
7. Carefully transfer 630 µl of the mixture from step 5 to the QIAamp® spin column WITHOUT moistening the rim of the column.
8. Centrifuge 1 minute at 6,000 x g. If the specimen has not cleared the filter after the first run, repeat centrifugation until the specimen has cleared the filter. Discard the collection tube containing the flow through.
9. For each specimen and control, place the QIAamp® spin column into a second, clean 2 mL collection tube (from the QIAamp® Viral RNA Mini Kit). Add the remaining mixture from step 5 to the respective spin column WITHOUT moistening the rim of the column. Pay special attention to add the remaining mixture to the correct column!

10. Centrifuge 1 minute at 6,000 x g. If the specimen has not cleared the filter after the first run, repeat centrifugation until the specimen has cleared the filter. Discard the collection tube containing the flow through.
11. For each specimen and control, place the QIAamp® spin column into another, clean 2 mL collection tube (from the QIAamp® Viral RNA Mini Kit) and add 500 µl of Buffer AW1. Discard the tube containing the filtrate from the previous step.
12. Centrifuge 1 minute at 6,000 x g. If the buffer has not cleared the filter after 1 minute, repeat centrifugation until buffer has cleared the filter.
13. Place each QIAamp® spin column into a fourth clean 2 mL collection tube (from the QIAamp® Viral RNA Mini Kit). Carefully open the QIAamp® spin column and add 500 µl of Buffer AW2.
14. Centrifuge at full speed (approx. 14,000 x g) for 3 minutes. Discard the tube containing the filtrate from the previous step.
15. To eliminate any possible Buffer AW2 carryover, place the QIAamp® spin column into a new collection tube, discard the old collection tube, and centrifuge at full speed (approx. 14,000 x g) for 3 minutes.
16. Place the QIAamp® spin column in a clean, clearly labelled 1.5 mL RNase-free microcentrifuge tube (not provided). Discard the old collection tube containing the filtrate.
17. Carefully open the QIAamp® spin column and add 60 µl of Buffer AVE that has been equilibrated to room temperature. Close the cap, and incubate at room temperature for 1 minute. Centrifuge at 6,000 x g for 1 minute, RNA is now present in the eluate and ready to test. Store extracted specimens and controls at 2-8°C until PCR master mixes are prepared.

Extracted specimens should be tested with the RealStar® Zika Virus RT-PCR Kit U.S. within 6 hours of completing the extraction process. Residual unextracted specimens should be stored at 2-8°C while testing is in progress. Long-term storage of extracted specimens (>6 hours) should be at -20°C. Minimize (not to exceed 3) repeated freeze-thaw cycles.

NOTES

⚠ ***Never add the Internal Control directly to the specimen!***

⚠ ***The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.***

⚠ ***Ethanol is a strong inhibitor in real-time PCR. Make sure to eliminate any traces of ethanol prior to elution of the nucleic acid.***

For additional information and technical support regarding pre-treatment and sample preparation please contact our Technical Support:

e-mail: **support@altona-diagnostics.com**
phone USA: **+1 415 777 1712**
phone headquarter Hamburg: **+49-(0)40-5480676-0**

10.2 Master Mix Setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

The RealStar® Zika Virus RT-PCR Kit U.S. contains a heterologous Internal Control (IC), which serves as a control of the sample preparation procedure (nucleic acid extraction) and as a RT-PCR inhibition control.

- The Master Mix is set up according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Volume Master Mix	**20 µl**	**240 µl**

10.3 Reaction Setup

- Pipette 20 µl of the Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
- Add 1 µl of the IC into the wells that will be used for the Negative Control (PCR grade water) and the Positive Control. Do not add additional IC template into the wells that will be used with any sample or control which has been extracted previously and already contains IC.
- Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (Positive or Negative Control).
- Make sure that the Positive Control and at least one Negative Control (PCR grade water) is used per run.

- Thoroughly mix the samples and controls with the Master Mix by up and down pipetting.
- Close the 96-well reaction plate with an appropriate optical adhesive film, the reaction tubes with appropriate lids.
- Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1000 x g (~ 3000 rpm).

Reaction Setup	
Master Mix	20 µl
Sample or Control	10 µl
Total Volume	**30 µl**

11. Programming of Real-time PCR Instruments

For basic information regarding the setup and programming of the different real-time PCR instruments, please refer to the manual of the respective instrument.

For detailed programming instructions regarding the use of the RealStar® Zika Virus RT-PCR Kit U.S. on the LightCycler®480 Instrument II (Roche), CFX96™ Real-Time PCR Detection System (BIO-RAD), CFX96™ Deep Well Real-Time PCR Detection System (BIO-RAD), ABI Prism® 7500 SDS and ABI Prism® 7500 Fast SDS (Applied Biosystems), Rotor-Gene® 6000 (Corbett Research) and Rotor-Gene® Q 5/6 plex/MDx Platform (QIAGEN), please refer to chapter 11.4 “Special remarks on the setup of authorized real-time PCR instruments”. For further questions please contact our Technical Support (see section 16).

11.1 Settings

- Define the following settings:

Settings	
Reaction Volume	30 µl
Ramp Rate	Default
Passive Reference	ROX™

11.2 Fluorescent Detectors (Dyes)

- Define the fluorescent detectors (dyes) or targets, respectively:

Detection	Detector Name	Reporter	Quencher
Zika virus specific RNA	Zika virus	FAM™	(None)
Internal Control	IC	JOE™/VIC™	(None)

11.3 Temperature Profile and Dye Acquisition

- Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature	Time
Reverse Transcription	Hold	1	-	55 °C	20:00 min
Denaturation	Hold	1	-	95 °C	2:00 min
Amplification	Cycling	45	-	95 °C	0:15 min
			√	55 °C	0:45 min
			-	72 °C	0:15 min

11.4 Special remarks on the setup of authorized real-time PCR instruments

Please find below special remarks on the setup of LightCycler® 480 Instrument II (Roche), CFX96™ Real-Time PCR Detection System (BIO-RAD), CFX96™ Deep Well Real-Time PCR Detection System (BIO-RAD), ABI Prism® 7500 SDS and ABI Prism® 7500 Fast SDS (Applied Biosystems), Rotor-Gene® 6000 (Corbett Research) and Rotor-Gene® Q 5/6 plex Platform (QIAGEN).

11.4.1 Special remarks on the setup of the LightCycler® 480 Instrument II

1. In the “Experiment settings”, select “Detection Format: Dual Color Hydrolysis Probe / UPL Probe”.
2. Make sure by checking the “Customize” field that the setting shown for the “Filter Combinations” are “FAM™ (465-510)” and “VIC™/HEX/Yellow555 (533-580)”.

11.4.2 Special remarks on the setup of the ABI Prism® 7500 SDS

Go to “Plate Setup”, “Define Targets and Samples”, “Assign Targets and Samples”:

1. Select the whole plate.
2. Click the assign-boxes for both targets. The targets should appear in the wells in the plate layout.
3. Make sure to choose “none” in the “Select the dye to use as the passive reference” (default setting is “ROX™”).

11.4.3 Special remarks on the setup of the ABI Prism® 7500 SDS Fast

The same settings for “Plate Setup” as for the ABI Prism® 7500 SDS apply (see above). For the Fast version, go to “Experiment properties”. The ramp speed has to be set to “Standard (~2 hours to complete a run)”. The RealStar® Zika Virus RT-PCR Kit U.S. is not compatible with the fast cycling conditions and the increased ramp rates.

11.4.4 Special remarks on the setup of the CFX96™ Real-Time PCR Detection System and the CFX96™ Deep Well Real-Time PCR Detection System

Open the “Plate Editor” window and select all wells of the 96 well-plate. Click “Select Fluorophores”. For “Channel 1” check the box behind FAM™ and for “Channel 2” check the box behind VIC™. Assign samples to the wells by selecting the appropriate “Sample Type “and afterwards “Load” FAM™ and VIC™ to the wells. The target name of FAM™ should be set to “Zika virus” and the target name of VIC to “Internal Control”.

11.4.5. Special remarks on the setup of the Rotor-Gene® 6000 and Rotor-Gene® Q 5/6 plex/MDx Platform

Chose the 72-Well-Rotor and the appropriate reaction volume. The Gain optimization should be performed before 1st acquisition.

12. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the manual of the respective instrument.
For questions regarding data analysis of the RealStar® Zika Virus RT-PCR Kit U.S. on authorized real-time PCR instruments please contact our Technical Support (see section 16).

12.1 Validity of Diagnostic Test Runs

12.1.1 Valid Diagnostic Test Run

For a **valid** diagnostic test run, the following control conditions must be met:

Control ID	FAM™ Detection Channel (Zika virus)	JOE™/VIC™ Detection Channel (Internal Control)
Positive Control	POSITIVE	POSITIVE
Negative Control (PCR grade water)	NEGATIVE	POSITIVE
Negative Process Control (NPC)	NEGATIVE	POSITIVE

12.1.2 Invalid Diagnostic Test Run

A diagnostic test run is **invalid**, (i) if the run has not been completed or (ii) if any of the control conditions for a **valid** diagnostic test run are not met.

In the case of an **invalid** diagnostic test run, repeat testing by using the remaining purified nucleic acids or start from the original samples again.

If a test run is **repeatedly invalid** please contact our Technical Support (see section 16).

12.2 Interpretation of Results

Sample ID	FAM™ Detection Channel (Zika virus)	JOE™/VIC™ Detection Channel (Internal Control)	Result Interpretation
A	POSITIVE	POSITIVE*	Zika virus specific RNA detected. All Zika virus specific RNA detected results must be reported to the appropriate Public Health agency.
B	NEGATIVE	POSITIVE	Zika virus specific RNA not detected. Sample does not contain detectable amounts of Zika virus specific RNA.
C	NEGATIVE	NEGATIVE	PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

* Detection of the Internal Control in the JOE™/VIC™ channel is not required for positive results in the FAM™ detection channel. A high Zika virus RNA load in the sample can lead to a reduced or absent Internal Control signal.

NOTE

> ⚠ ***Any positive specimen result (urine or serum) is considered a positive diagnosis of Zika virus infection. Zika virus specific RNA detected results must be reported to the appropriate Public Health agency. Please refer to the CDC website for the most update information on patient follow up. http://www.cdc.gov/zika/hc-providers/clinical-guidance.html.***

13. Analytical Performance Evaluation

13.1 Analytical Sensitivity - Serum Samples

Estimation of the Limit of Detection (LoD):
Serial dilutions of Zika virus strain H/PF/2013 (stock concentration TCID50/mL: $10^{6.82}$) obtained from the European Virus Archive (Marseille, France) were prepared. Using qRT-PCR and quantified *in vitro* transcribed RNA the number of Zika virus genome equivalents (geq) per mililiter stock was determined to be 1.26E+09.

For nucleic acid extraction, 126 µL of pooled serum were combined with 560 µL AVL buffer, containing 6 µL of the Internal Control (provided with the RealStar® Zika Virus RT-PCR Kit U.S.) and spiked with 14 µL diluted Zika virus stock. The final mix was subjected to the extraction procedure following the manufacturer's instructions for the QIAamp® Viral RNA Mini Kit (QIAGEN). Elution was performed in 60 µL AVE buffer. Each sample was extracted in triplicate and tested with the RealStar® Zika Virus RT-PCR Kit U.S. on the CFX96™ Deep Well Real-Time PCR Detection System (BIO-RAD). The lowest concentration at which all three replicates were tested positive was treated as the tentative LoD. The results can be found in Table 2:

Table 2: Determination of the tentative LoD for the RealStar® Zika Virus RT-PCR Kit U.S.

Target	Concentration geq/mL	Call rate	Replicate 1 Ct (FAM™)	Replicate 2 Ct Ct (FAM™)	Replicate 3 Ct (FAM™)
Zika virus (strain H/PF/2013)	2515.71	3/3	33.52	33.76	33.87
	795.61	3/3	35.13	35.36	34.85
	251.62	3/3	36.98	35.92	35.86
	79.57	2/3	42.00	-	36.05
	25.17	1/3	36.74	-	-
	7.96	1/3	-	37.49	-
	2.52	0/3	-	-	-

The RealStar® Zika Virus RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the CFX96™ Deep Well Real-Time PCR Detection System detected 3/3 replicates with a concentration of 251.62 geq/mL serum.

Confirmation of the Limit of Detection (LoD):
Based on the tentative LoD, diluted Zika virus stock was spiked into 20 individual serum samples to a final concentration of 251.62 geq/mL. Nucleic acids were extracted with the QIAamp® Viral RNA Mini Kit (QIAGEN) as described above. The obtained eluates were tested with the RealStar® Zika Virus RT-PCR Kit U.S. on the CFX96™ Deep Well Real-Time PCR Detection System (BIO-RAD). The results can be found in Table 3:

Table 3: LoD confirmation on the CFX96™ Deep Well Real-Time PCR Detection System

Zika virus concentration = 251.62 geq/mL			
Specimen	**Pos/Neg**	**Ct (FAM™)**	**Ct (VIC™)**
1	+	36.84	31.55
2	+	38.29	32.16
3	+	36.07	31.71
4	+	35.18	31.32
5	+	36.95	31.60
6	+	36.22	31.71
7	+	35.48	32.06
8	+	35.12	32.04
9	+	36.05	31.49
10	+	36.07	31.89
11	+	35.77	31.69
12	-	-	32.68
13	+	36.51	32.17
14	+	36.21	32.32
15	+	34.96	31.66
16	+	36.08	32.18
17	+	35.97	32.05
18	+	35.47	31.41
19	+	36.07	31.86
20	+	36.15	31.87
Statistics	Mean Ct (n=19)	36.08	31.87
	SD	0.76	0.34
	CV%	2.09	1.06
	Result	19/20	

The RealStar® Zika Virus RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the CFX96™ Deep Well Real-Time PCR Detection System detected 19/20 replicates at a concentration of 251.62 geq/mL. Therefore, the confirmed LoD is 251.62 geq/mL serum.

***Confirmation of the Limit of Detection (LoD)* for additional real-time PCR instruments:**

Based on the results obtained from the LoD confirmation using the CFX96™ Deep Well Real-Time PCR Detection System (BIO-RAD), diluted Zika virus stock was spiked into 20 individual serum samples to a final concentration of 251.62 geq/mL. Nucleic acids were extracted with the QIAamp® Viral RNA Mini Kit (QIAGEN) and tested with the RealStar® Zika Virus RT-PCR Kit U.S. on the following additional real-time PCR instruments:

1. Rotor-Gene® Q (QIAGEN)
2. ABI Prism® 7500 SDS (Applied Biosystems)
3. LightCycler® 480 Instrument II (Roche)

The results can be found in Tables 4, 5 and 6:

Table 4: LoD confirmation on the Rotor-Gene® Q (QIAGEN)

Zika virus concentration = 251.62 geq/mL			
Specimen	**Pos/Neg**	**Ct (FAM™)**	**Ct (JOE™)**
1	34.68	30.34	31.55
2	36.10	30.74	32.16
3	35.74	30.57	31.71
4	34.62	30.05	31.32
5	34.93	30.29	31.60
6	35.12	30.58	31.71
7	35.29	30.60	32.06
8	35.08	30.63	32.04
9	34.38	30.32	31.49
10	36.47	30.42	31.89
11	34.33	30.29	31.69
12	36.78	31.39	32.68
13	35.76	31.02	32.17
14	34.99	30.89	32.32
15	34.59	30.36	31.66
16	35.40	30.83	32.18
17	34.58	30.64	32.05
18	34.88	30.27	31.41
19	35.99	30.29	31.86
20	34.67	30.32	31.87
Statistics	Mean Ct (n=20)	35.22	30.54
	SD	0.71	0.32
	CV%	2.01	1.04
	Result	20/20	

At the concentration of 251.62 geq/mL 20/20 replicates were detected positive and thereby confirm the LoD to be 251.62 geq/mL serum for the RealStar® Zika Virus RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the Rotor-Gene® Q (QIAGEN).

Table 5: LoD confirmation on ABI Prism® 7500 SDS (Applied Biosystems)

Zika virus concentration = 251.62 geq/mL			
Specimen	**Pos/Neg**	**Ct (FAM™)**	**Ct (JOE™)**
1	+	36.18	30.20
2	+	35.11	30.67
3	+	35.02	30.38
4	+	35.49	29.88
5	+	34.86	30.35
6	+	35.37	30.09
7	+	35.61	30.33
8	+	34.67	30.28
9	+	34.85	30.12
10	+	35.61	29.91
11	+	34.82	30.05
12	+	35.78	31.07
13	+	34.78	30.95
14	+	36.43	30.75
15	+	34.25	30.19
16	+	35.33	30.57
17	+	34.86	30.31
18	+	36.58	30.00
19	+	35.10	30.13
20	+	34.74	30.22
Statistics	Mean Ct (n=20)	35.27	30.32
	SD	0.62	0.33
	CV%	1.74	1.08
	Result	20/20	

At the concentration of 251.62 geq/mL 20/20 replicates were detected positive and thereby confirm the LoD to be 251.62 geq/mL serum for the RealStar® Zika Virus RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the ABI Prism® 7500 SDS (Applied Biosystems).

Table 6: LoD confirmation on LightCycler® 480 Instrument II (Roche)

Zika virus concentration = 251.62 geq/mL			
Specimen	**Pos/Neg**	**Ct (FAM™)**	**Ct (JOE™)**
1	+	35.55	30.93
2	+	36.93	31.28
3	+	35.84	30.73
4	+	35.49	30.60
5	+	36.89	30.79
6	+	36.48	30.81
7	+	35.88	30.80
8	+	35.37	30.89
9	+	35.46	30.59
10	+	35.85	31.01
11	+	35.90	30.55
12	+	36.33	31.74
13	+	36.30	31.28
14	+	37.59	31.22
15	+	35.74	30.77
16	+	36.18	31.04
17	+	36.48	31.13
18	+	36.00	30.66
19	+	36.48	30.78
20	+	35.82	30.78
Statistics	Mean Ct (n=20)	36.13	30.92
	SD	0.57	0.29
	CV%	1.57	0.95
	Result	20/20	

At the concentration of 251.62 geq/mL 20/20 replicates were detected positive and thereby confirm the LoD to be 251.62 geq/mL serum for the RealStar® Zika Virus RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the LightCycler® 480 Instrument II (Roche).

13.2 Analytical Sensitivity - Urine Samples

Estimation of the Limit of Detection (LoD):
Serial dilutions of Zika virus strain H/PF/2013 (stock concentration TCID50/mL: $10^{6.82}$) obtained from the European Virus Archive (Marseille, France) were prepared. Using qRT-PCR and quantified *in vitro* transcribed RNA the number of Zika virus genome equivalents (geq) per mililiter stock was determined to be 1.26E+09.

For nucleic acid extraction, 126 µL of pooled urine were combined with 560 µL AVL buffer, containing 6 µL of the Internal Control (provided with the RealStar® Zika Virus RT-PCR Kit U.S.) and spiked with 14 µL diluted Zika virus stock. The final mix was subjected to the extraction procedure following the manufacturer's instructions for the QIAamp® Viral RNA Mini Kit (QIAGEN). Elution was performed in 60 µL AVE buffer. Each sample was extracted in triplicate and tested with the RealStar® Zika Virus RT-PCR Kit U.S. on the CFX96™ Deep Well Real-Time PCR Detection System (BIO-RAD). The lowest concentration at which all three replicates were tested positive was treated as the tentative LoD. The results can be found in Table 7:

Table 7: Determination of the tentative LoD for the RealStar® Zika Virus RT-PCR Kit U.S.

Target	Concentration geq/mL	Call rate	Replicate 1 Ct (FAM™)	Replicate 2 Ct (FAM™)	Replicate 3 Ct (FAM™)
Zika virus (strain H/PF/2013)	2515.71	3/3	33.15	33.34	33.11
	795.61	3/3	34.10	33.83	35.07
	251.62	3/3	35.54	35.64	35.27
	79.57	3/3	36.78	37.41	36.40
	25.17	2/3	38.38	37.72	-
	7.96	1/3	-	-	38.22
	2.52	0/3	-	-	-

The RealStar® Zika Virus RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the CFX96™ Deep Well Real-Time

PCR Detection System detected 3/3 replicates with a concentration of 79.57 geq/mL urine.

Confirmation of the Limit of Detection (LoD):

Based on the tentative LoD, diluted Zika virus stock was spiked into 20 individual urine samples to a final concentration of 79.57 geq/mL. Nucleic acids were extracted with the QIAamp® Viral RNA Mini Kit (QIAGEN) as described above. The obtained eluates were tested with the RealStar® Zika Virus RT-PCR Kit U.S. on the CFX96™ Deep Well Real-Time PCR Detection System (BIO-RAD). The results can be found in Table 8:

Table 8: LoD confirmation on CFX96™ Deep Well Real-Time PCR Detection System

Zika virus concentration = 79.57 geq/mL			
Specimen	**Pos/Neg**	**Ct (FAM™)**	**Ct (VIC™)**
1	+	36.72	30.77
2	+	36.34	31.06
3	+	37.21	31.07
4	+	36.41	31.21
5	+	37.89	30.59
6	-	-	31.21
7	+	36.12	31.25
8	+	36.25	31.43
9	+	38.03	30.64
10	+	37.36	31.11
11	+	38.08	30.99
12	+	37.26	30.95
13	+	36.30	31.27
14	+	35.75	30.74
15	+	36.68	31.01
16	+	39.07	31.08
17	+	37.17	31.67
18	+	35.79	31.04
19	+	38.01	31.44
20	+	37.17	31.05
Statistics	Mean Ct (n=19)	37.03	31.08
	SD	0.90	0.27
	CV%	2.42	0.87
	Result	19/20	

The RealStar® Zika Virus RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the CFX96™ Deep Well Real-Time PCR Detection System detected 19/20 replicates at a concentration of 79.57 geq/mL. Therefore, the confirmed LoD is 79.57 geq/mL urine.

***Confirmation of the Limit of Detection (LoD)* for additional real-time PCR instruments:**

Based on the results obtained from the LoD confirmation using the CFX96™ Deep Well Real-Time PCR Detection System (BIO-RAD), diluted Zika virus stock was spiked into 20 individual urine samples to a final concentration of 79.57 geq/mL. Nucleic acids were extracted with the QIAamp® Viral RNA Mini Kit (QIAGEN) and tested with the RealStar® Zika Virus RT-PCR Kit U.S. on the following additional real-time PCR instruments:

1. Rotor-Gene® Q (QIAGEN)
2. ABI Prism® 7500 SDS (Applied Biosystems)
3. LightCycler® 480 Instrument II (Roche)

The results can be found in Tables 9,10 and 11:

Table 9: LoD confirmation on the Rotor-Gene® Q (QIAGEN)

Zika virus concentration = 79.57 geq/mL			
Specimen	**Pos/Neg**	**Ct (FAM™)**	**Ct (JOE™)**
1	+	36.45	29.66
2	+	35.47	30.2
3	+	36.03	30.37
4	+	35.70	30.08
5	+	35.97	29.53
6	+	37.38	30.41
7	-	-	30.03
8	+	37.34	30.23
9	+	35.36	29.49
10	+	36.40	30.10
11	+	35.57	29.83
12	+	36.52	29.74
13	+	37.59	30.21
14	+	35.49	29.53
15	+	36.12	29.89
16	+	36.53	29.83
17	+	36.76	30.73
18	+	36.55	29.79
19	+	36.19	30.39
20	+	37.39	29.79
Statistics	Mean Ct (n=19)	36.36	29.99
	SD	0.70	0.34
	CV%	1.92	1.13
	Result	19/20	

At the concentration of 79.57 geq/mL 19/20 replicates were detected positive and thereby confirm the LoD to be 79.57 geq/mL urine for the RealStar® Zika Virus RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the Rotor-Gene® Q (QIAGEN).

Table10: LoD confirmation on ABI Prism® 7500 SDS (Applied Biosystems)

Zika virus concentration = 79.57 geq/mL			
Specimen	**Pos/Neg**	**Ct (FAM™)**	**Ct (JOE™)**
1	+	36.11	29.48
2	+	36.63	29.97
3	+	35.76	29.94
4	+	37.29	29.85
5	+	36.54	29.56
6	+	37.37	29.86
7	+	36.36	30.03
8	+	37.27	30.11
9	+	36.05	29.45
10	+	36.65	30.06
11	+	37.33	29.86
12	+	35.88	29.53
13	+	36.09	30.02
14	+	37.58	29.51
15	+	37.36	29.86
16	+	36.07	29.80
17	+	36.50	30.23
18	+	37.36	29.81
19	+	35.68	30.20
20	+	35.74	29.74
Statistics	Mean Ct (n=20)	36.58	29.84
	SD	0.65	0.24
	CV%	1.79	0.8
	Result	20/20	

At the concentration of 79.57 geq/mL 20/20 replicates were detected positive and thereby confirm the LoD to be 79.57 geq/mL urine for the RealStar® Zika Virus RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the ABI Prism® 7500 SDS (Applied Biosystems).

Table 11: LoD confirmation on LightCycler® 480 Instrument II (Roche)

Zika virus concentration = 79.57 geq/mL			
Specimen	**Pos/Neg**	**Ct (FAM™)**	**Ct (JOE™)**
1	+	37.29	30.15
2	+	37.03	30.34
3	+	37.67	30.48
4	+	36.83	30.42
5	+	36.58	30.02
6	+	36.90	30.69
7	+	37.50	30.66
8	+	38.63	30.71
9	+	36.74	30.10
10	+	37.95	30.54
11	+	37.13	30.52
12	+	38.12	30.29
13	+	36.53	30.54
14	+	37.41	30.19
15	+	37.16	30.43
16	+	36.70	30.61
17	+	37.79	30.86
18	+	37.27	30.41
19	+	40.00	30.86
20	+	38.42	30.35
Statistics	Mean Ct (n=20)	37.48	30.46
	SD	0.84	0.24
	CV%	2.25	0.78
	Result	20/20	

At the concentration of 79.57 geq/mL 20/20 replicates were detected positive and thereby confirm the LoD to be 79.57 geq/mL urine for the RealStar® Zika Virus RT-PCR Kit U.S. in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the LightCycler® 480 Instrument II (Roche).

13.3 Analytical Specificity

13.3.1 Reactivity

The analytical specificity of the RealStar® Zika Virus RT-PCR Kit U.S. is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligonucleotides were checked by sequence comparison analysis against publicly available sequences to ensure that all relevant Zika virus strains and isolates will be detected.

Reactivity of the RealStar® Zika Virus RT-PCR Kit U.S. was evaluated by wet testing for additional isolates of Zika virus as shown in the Table 12:

Table 12: Reactivity (wet testing): RealStar® Zika Virus RT-PCR Kit U.S.

Zika Virus Strain	Sample Type	Concentration	Ct (FAM™)
MR766	Zika virus cell culture supernatant	408 geq/mL	37.30
ArD142623	*In vitro* transcribed RNA	320 geq/mL	38.32
MRS_OPY_Martinique_PaRi_2015	Freeze dried Zika virus	161 geq/mL	37.71

In addition to the wet testing reactivity of the RealStar® Zika Virus RT-PCR Kit U.S. was evaluated *in silico* by sequence comparison analysis against publicly available Zika virus sequences. The sequence identity of the strains and isolates included in the analysis with strain H/PF/2013 (used for LoD determination) in the binding region of the primers and probe included in the RealStar® Zika Virus RT-PCR Kit U.S. is shown in Table 13:

Table 13: Reactivity (*in silico* analysis): RealStar® Zika Virus RT-PCR Kit U.S.

Zika Virus Isolate/Strain	Sequence identity with strain H/PF/2013 in binding region of the primers and probe included in the RealStar® Zika Virus RT-PCR Kit U.S.
Isolate Zika virus/H. sapiens-tc/ PHL/2012/ CPC-0740	98%
Isolate ARB7701	95%
Isolate ARB13565	95%
Isolate ARB15076	97%
Strain Natal RGN	100%
Isolate Zika virus/H. sapiens-tc/ THA/2014/SV0127	100%
Strain PRVABC59	100%
Strain Haiti/1225/2014	100%
Isolate VE_Ganxian	98%
Strain ZikaSPH2015	100%
Isolate Brazil-ZKV2015	100%
Strain BeH819015	100%
Strain BeH815744	100%
StrainBeH819966	100%
Strain BeH818995	100%
Isolate SSABR1	100%
Strain MRS_OPY_Martinique_PaRi_2015	100%
Isolate Z1106033	100%
Isolate FSS13025	100%
Isolate P6-740	100%
Isolate GD01	100%
Strain ArB1362	97%
Strain 8375	100%
Strain 103344	100%

Table 13: (continuation)

Zika Virus Isolate/Strain	Sequence identity with strain H/PF/2013 in binding region of the primers and probe included in the RealStar® Zika Virus RT-PCR Kit U.S.
Strain PLCal_ZV from Canada	100%
Isolate Si323	100%
Isolate Si322	100%
Isolate ArD_41519	97%
Isolate IbH_30656	95%
Strain MR 766	97%
Strain ArD7117	97%
Strain ArD142623	87%
Strain ArD128000	97%
Strain ArD158095	97%
Strain ArD158084	97%
Strain ArD157995	97%

13.3.2 Cross reactivity

To evaluate the analytical specificity of the RealStar® Zika Virus RT-PCR Kit U.S. with regards to cross reactivity, genomic RNA or DNA from different viruses related to Zika virus as well as to other pathogens was tested (for details see Table 14 below). The genomic RNA/DNA was extracted from cell culture supernatant or clinical specimens provided by the Bernhard Nocht Institute for Tropical Medicine (BNITM, Hamburg, Germany). All samples were analyzed with the RealStar® Zika Virus RT-PCR Kit U.S. on the CFX96™ Deep Well Real-Time PCR Detection System (BIO-RAD).

Table 14: Cross Reactivity (wet testing): RealStar® Zika Virus RT-PCR Kit U.S.

Pathogen	FAM™ channel (Zika virus)	VIC™ channel (Internal Control)
Japanese encephalitis virus	Negative	Positive
St. Louis encephalitis virus	Negative	Positive
West Nile virus	Negative	Positive
Yellow fever virus	Negative	Positive
Murray Valley encephalitis virus	Negative	Positive
SEBOV	Negative	Positive
ZEBOV	Negative	Positive
MARV	Negative	Positive
Dengue virus serotype 1	Negative	Positive
Dengue virus serotype 2	Negative	Positive
Dengue virus serotype 3	Negative	Positive
Dengue virus serotype 4	Negative	Positive
Chikungunya virus	Negative	Positive
Parvovirus B19	Negative	Positive
Plasmodium falciparum	Negative	Positive

No cross reactivity of the RealStar® Zika Virus RT-PCR Kit U.S. with genomic RNA/DNA of the selected pathogens was observed. All samples tested generated a positive Internal Control signal in the VIC™ channel, whereas no signal was observable in the Zika virus target specific (FAM™) channel.

For the pathogens included in the wet testing as well as for additional pathogens with limited or no availability, an *in silico* analysis was done showing that cross reactivity is unlikely to occur.

In silico analysis included genomic RNA/DNA sequences of the viruses and organisms listed in Table 15:

Table 15: Cross reactivity (*in silico* analysis): RealStar® Zika Virus RT-PCR Kit U.S.

Flaviviruses	Other Pathogens
Dengue virus 1, 2, 3 and 4	Eastern Equine Encephalitis Virus
Yellow fever virus	Western Equine Encephalitis Virus
Yellow fever vaccine strain	Ross River virus
West Nile virus	Barmah Forest virus
St. Louis encephalitis virus	O'nyong-nyong virus
Japanese encephalitis virus	Sindbis virus
Spondweni virus	Tonate virus
Hepatitis C virus	Una virus
	Measles virus
	Rubella virus
	Enterovirus
	Adenovirus
	Hepatitis B virus
	Human immunodeficiency virus
	Varicella Zoster virus
	Cytomegalovirus
	Epstein-Barr virus
	Rickettsia sp.
	Borrelia burgdorferi
	Group A *Streptococcus*
	Leptospira sp.
	Plasmodium sp.
	Plasmodium vivax
	Trypanosoma cruzi
	Schistosoma sp.
	Hepatitis A virus vaccine - BIOVAC-A brand
	Salmonella typhi vaccine (Typhoid - Ty21a vaccine)

The sequences of the primers included in the RealStar® Zika Virus RT-PCR Kit U.S. were blasted against the species specified in the Table above
The BLAST algorithms were set to: blastn; Max target sequences: 10,000; Expect threshold: 1,000; Word size 7; Match/mismatch scores: 1,-3; Gap Costs Existence: 5 Extension: 2.
All primers contained in the RealStar® Zika Virus RT-PCR Kit U.S. were blasted in all possible combinations. Hits were reviewed for potential formation of PCR product through binding of the primers in close proximity and with the right orientation to each other on target nucleic acid molecules. No constellation was found that could lead to undesired amplification of potentially cross-reacting target sequences.

13.3.3 Interfering Substances

Interference studies were not performed for the RealStar® Zika Virus RT-PCR Kit U.S., since the test uses conventional real-time RT-PCR and an established extraction method prior to testing (column based extraction).
The RealStar® Zika Virus RT-PCR Kit U.S. contains an Internal Control, which has to be added for each specimen tested to the nucleic acid extraction procedure. The Internal Control is reverse transcribed, amplified and detected in parallel to the Zika virus specific RNA and ensures the integrity of Zika virus specific real-time RT-PCR results by indicating potential RT-PCR inhibition.

14. Clinical Performance Evaluation

To evaluate the clinical performance of the RealStar® Zika Virus RT-PCR Kit U.S. a total of 208 clinical specimens from 153 patients with signs and symptoms of Zika virus infection (106 female (F) and 47 male (M)) were analyzed retrospectively in a blinded fashion. From the 208 samples tested 103 were serum and 105 were urine specimens.

For RNA extraction the QIAamp® Viral RNA Mini Kit (QIAGEN) was used. 140 µL of each urine or serum sample were combined with 560 µL AVL buffer, containing 6 µL of the Internal Control provided with the RealStar® Zika Virus RT-PCR Kit U.S.. The sample/AVL buffer mix was subjected to the extraction procedure following the manufacturer's instructions. Elution was performed in 60 µL AVE buffer.
Eluates were tested with the RealStar® Zika Virus RT-PCR Kit U.S. as well as with the real-time RT-PCR as described by Lanciotti et. al (Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, et al. (2008) Genetic and Serologic Properties of Zika Virus Associated with an Epidemic, Yap State, Micronesia, 2007; Emerg Infect Dis. 2008 Aug; 14(8): 1232–1239).
For the execution of the real-time RT-PCR described by Lanciotti et al. the following oligonucleotides; Table 16, have been used:

Table 16: Oligonucleotides used for real-time RT-PCR described by Lanciotti et al.

Primer/Probe	Sequence 5'->3'
ZIKV 1086	CCGCTGCCCAACACAAG
ZIKV 1162c	CCACTAACGTTCTTTTGCAGACAT
ZIKV 1107-FAM	AGCCTACCTTGACAAGCAGTCAGACACTCAA

Both tests, the RealStar® Zika Virus RT-PCR Kit U.S. as well as the real-time RT-PCR described by Lanciotti et al., have been performed on a CFX96™ Deep Well Real-Time PCR Detection System (BIO-RAD). Results generated for the different samples tested are shown in Table 17:

Table 17: Results from testing clinical samples

Patient ID	Gender	Days after Onset of Symptoms	Specimen Type	RealStar® Zika Virus RT-PCR Kit U.S.			Real-time RT-PCR described by Lanciotti et al.	
				Ct (FAM™)	Call	Ct (VIC™)	Ct (FAM™)	Call
1	F	2	Serum	-	-	29.88	-	-
		2	Urine	-	-	31.57	-	-
2	F	5	Urine	-	-	29.50	-	-
3	F	1	Serum	-	-	30.37	-	-
		1	Urine	-	-	29.49	-	-
4	F	1	Serum	-	-	30.32	-	-
		1	Urine	-	-	30.16	-	-
5	F	1	Serum	-	-	31.04	-	-
		1	Urine	-	-	30.10	-	-
6	F	3	Serum	-	-	30.77	-	-
		3	Urine	-	-	30.14	-	-
7	M	4	Serum	-	-	30.05	-	-
		4	Urine	-	-	31.21	-	-
8	M	0	Serum	-	-	29.61	-	-
		0	Urine	-	-	29.72	-	-
9	F	6	Urine	-	-	29.88	-	-
10	F	4	Serum	-	-	31.88	-	-
		4	Urine	-	-	29.83	-	-
11	F	0	Serum	-	-	31.33	-	-
		0	Urine	-	-	30.35	-	-
12	M	3	Serum	-	-	30.10	-	-
		3	Urine	-	-	30.34	-	-

Table 17: (continuation)

Patient ID	Gender	Days after Onset of Symptoms	Specimen Type	RealStar® Zika Virus RT-PCR Kit U.S.			Real-time RT-PCR described by Lanciotti et al.	
				Ct (FAM™)	Call	Ct (VIC™)	Ct (FAM™)	Call
13	F	4	Urine	33.03	+	29.92	33.85	+
14	F	2	Serum	33.53	+	29.84	32.73	+
15	M	5	Serum	-	-	30.53	-	-
		5	Urine	33.76	+	30.02	34.83	+
16	M	2	Serum	-	-	30.45	-	-
		2	Urine	-	-	29.87	-	-
17	F	3	Serum	30.12	+	31.45	29.68	+
18	F	3	Urine	-	-	29.81	-	-
19	F	2	Serum	-	-	29.60	-	-
		2	Urine	33.41	+	30.24	33.95	+
20	F	6	Urine	-	-	29.85	38.64	+
21	M	2	Serum	-	-	31.79	-	-
		2	Urine	-	-	29.96	-	-
22	M	1	Serum	-	-	32.04	-	-
		1	Urine	-	-	29.86	-	-
23	F	2	Serum	-	-	30.90	-	-
24	F	7	Urine	-	-	30.46	-	-
25	F	0	Serum	33.35	+	30.43	33.33	+
		3	Urine	31.93	+	31.23	32.81	+
26	F	3	Serum	-	-	30.81	-	-
		3	Urine	-	-	30.13	-	-
27	F	0	Urine	-	-	29.40	-	-
28	M	5	Serum	-	-	31.43	-	-
		5	Urine	-	-	30.49	-	-
29	F	7	Urine	-	-	30.09	-	-

Table 17: (continuation)

Patient ID	Gender	Days after Onset of Symptoms	Specimen Type	RealStar® Zika Virus RT-PCR Kit U.S.			Real-time RT-PCR described by Lanciotti et al.	
				Ct (FAM™)	Call	Ct (VIC™)	Ct (FAM™)	Call
30	M	1	Serum	-	-	32.57	-	-
		1	Urine	-	-	29.97	-	-
31	F	1	Serum	35.91	+	31.19	37.41	+
		1	Urine	30.14	+	30.06	30.05	+
32	M	2	Serum	-	-	30.49	-	-
		2	Urine	-	-	30.29	-	-
33	F	2	Serum	32.15	+	31.35	31.84	+
34	F	0	Urine	35.43	+	29.90	36.43	+
35	M	7	Urine	34.39	+	29.59	35.78	+
36	F	2	Serum	-	-	30.01	37.85	+
37	F	2	Urine	-	-	30.02	-	-
38	M	8	Urine	-	-	35.35	-	-
39	F	6	Serum	30.94	+	30.13	30.34	+
40	M	3	Serum	-	-	29.80	-	-
		3	Urine	-	-	30.76	-	-
41	M	3	Serum	-	-	30.36	-	-
		3	Urine	-	-	30.38	-	-
42	F	0	Serum	30.42	+	31.04	29.72	+
43	M	6	Urine	-	-	30.04	-	-
44	F	1	Serum	35.92	+	30.69	36.27	+
		1	Urine	35.16	+	29.77	38.48	+
45	M	6	Urine	-	-	30.06	-	-
46	M	4	Serum	-	-	30.98	-	-
		4	Urine	-	-	30.11	-	-
47	M	3	Urine	34.23	+	30.42	36.50	+

Table 17: (continuation)

Patient ID	Gender	Days after Onset of Symptoms	Specimen Type	RealStar® Zika Virus RT-PCR Kit U.S.			Real-time RT-PCR described by Lanciotti et al.	
				Ct (FAM™)	Call	Ct (VIC™)	Ct (FAM™)	Call
48	M	2	Urine	26.17	+	29.09	25.65	+
49	M	4	Serum	-	-	30.08	-	-
		4	Urine	-	-	30.23	-	-
50	F	6	Urine	-	-	29.91	-	-
51	F	2	Serum	35.04	+	32.35	34.74	+
52	F	3	Serum	33.48	+	31.29	33.18	+
53	M	5	Serum	-	-	29.69	-	-
		5	Urine	-	-	30.04	-	-
54	F	1	Serum	26.08	+	29.83	25.24	+
55	F	2	Urine	-	-	30.02	-	-
56	F	6	Serum	-	-	31.71	-	-
		6	Urine	-	-	31.11	-	-
57	F	7	Urine	-	-	30.10	-	-
58	F	4	Serum	-	-	32.75	-	-
		4	Urine	-	-	30.20	-	-
59	M	1	Serum	-	-	30.41	-	-
		1	Urine	-	-	29.87	-	-
60	M	0	Serum	-	-	29.62	-	-
		0	Urine	-	-	29.82	-	-
61	F	2	Serum	-	-	30.52	-	-
62	F	7	Urine	-	-	30.06	-	-
63	F	0	Serum	-	-	31.47	-	-
		0	Urine	-	-	29.74	-	-
64	F	7	Urine	28.03	+	30.03	28.68	+
65	F	2	Serum	37.79	+	29.33	38.15	+
		2	Urine	36.07	+	29.47	37.66	+

Table 17: (continuation)

Patient ID	Gender	Days after Onset of Symptoms	Specimen Type	RealStar® Zika Virus RT-PCR Kit U.S.			Real-time RT-PCR described by Lanciotti et al.	
				Ct (FAM™)	Call	Ct (VIC™)	Ct (FAM™)	Call
66	F	2	Serum	33.06	+	31.45	33.21	+
67	F	4	Serum	30.66	+	29.53	30.19	+
68	M	1	Serum	-	-	30.79	-	-
69	M	2	Serum	35.08	+	31.68	35.14	+
70	F	3	Urine	-	-	30.39	39.17	+
71	F	1	Serum	-	-	29.83	-	-
72	M	1	Serum	36.58	+	31.89	-	-
73	F	3	Urine	33.36	+	30.55	32.98	+
74	F	4	Serum	-	-	30.94	-	-
		4	Urine	-	-	30.92	-	-
75	F	3	Serum	35.03	+	29.82	35.30	+
76	M	3	Serum	34.06	+	29.57	34.01	+
77	F	3	Urine	35.02	+	31.02	34.66	+
78	F	6	Serum	32.00	+	30.08	32.03	+
		6	Urine	33.55	+	30.06	35.52	+
79	F	2	Serum	34.99	+	29.65	36.29	+
		2	Urine	26.59	+	29.72	26.94	+
80	M	3	Serum	31.93	+	29.78	31.17	+
		3	Urine	37.78	+	32.00	-	-
81	F	4	Serum	35.07	+	31.86	35.32	+
		4	Urine	34.51	+	30.83	36.42	+
82	F	1	Serum	33.51	+	29.88	33.09	+
83	F	0	Serum	26.83	+	33.66	26.55	+
		8	Urine	19.37	+	-*	17.88	+
84	F	3	Urine	34.83	+	30.38	35.38	+
85	M	5	Urine	33.31	+	30.25	33.31	+

Table 17: (continuation)

Patient ID	Gender	Days after Onset of Symptoms	Specimen Type	RealStar® Zika Virus RT-PCR Kit U.S.			Real-time RT-PCR described by Lanciotti et al.	
				Ct (FAM™)	Call	Ct (VIC™)	Ct (FAM™)	Call
86	M	6	Urine	-	-	30.52	-	-
87	M	3	Serum	35.07	+	29.62	34.18	+
		3	Urine	31.01	+	30.34	30.48	+
88	F	6	Serum	-	-	29.54	-	-
		6	Urine	28.58	+	30.67	28.01	+
89	F	3	Serum	30.87	+	29.74	29.98	+
90	F	2	Serum	28.58	+	29.51	27.77	+
91	F	1	Serum	33.71	+	31.35	33.66	+
92	F	3	Urine	35.60	+	30.03	38.29	+
93	M	3	Serum	36.78	+	29.61	37.92	+
94	M	6	Urine	-	-	30.34	38.81	+
95	F	3	Serum	37.60	+	30.86	37.61	+
96	F	5	Urine	33.77	+	30.35	34.09	+
97	M	3	Serum	28.47	+	29.75	27.18	+
98	F	2	Serum	32.09	+	32.02	31.38	+
		2	Urine	37.64	+	31.16	38.33	+
99	F	1	Urine	33.47	+	30.57	35.44	+
100	F	4	Urine	-	-	30.31	-	-
101	M	7	Urine	-	-	30.30	-	-
102	F	0	Serum	-	-	32.62	-	-
103	F	3	Urine	33.63	+	30.30	33.45	+
104	F	2	Serum	36.05	+	32.30	36.08	+
		2	Urine	37.63	+	30.10	38.13	+
105	F	0	Serum	33.25	+	31.81	33.38	+
106	F	1	Urine	34.63	+	29.89	35.41	+
107	F	2	Serum	37.96	+	32.13	39.51	+

Table 17: (continuation)

Patient ID	Gender	Days after Onset of Symptoms	Specimen Type	RealStar® Zika Virus RT-PCR Kit U.S.			Real-time RT-PCR described by Lanciotti et al.	
				Ct (FAM™)	Call	Ct (VIC™)	Ct (FAM™)	Call
108	F	5	Serum	33.81	+	29.98	33.68	+
		5	Urine	35.89	+	30.31	36.57	+
109	F	1	Serum	-	-	30.38	-	-
110	M	7	Urine	-	-	30.61	-	-
111	F	1	Serum	37.07	+	31.19	38.54	+
112	F	2	Urine	35.76	+	29.94	-	-
113	F	2	Serum	31.13	+	31.50	30.62	+
114	M	1	Urine	-	-	29.83	-	-
115	F	5	Serum	-	-	30.30	36.89	+
		5	Urine	31.12	+	30.35	30.34	+
116	F	2	Serum	35.75	+	30.80	37.02	+
117	F	3	Urine	37.90	+	30.37	-	-
118	F	1	Serum	35.27	+	33.39	35.16	+
119	F	3	Serum	35.41	+	32.99	36.20	+
120	F	3	Urine	36.50	+	29.81	-	-
121	M	6	Urine	28.27	+	30.13	28.31	+
122	M	3	Serum	30.27	+	29.45	29.05	+
123	M	1	Serum	25.15	+	28.89	24.59	+
124	F	3	Urine	34.03	+	30.57	35.76	+
125	F	5	Urine	27.22	+	29.90	27.75	+
126	F	3	Urine	34.14	+	30.02	35.86	+
		1	Urine	37.81	+	30.20	-	-
127	F	2	Serum	27.47	+	29.41	27.38	+
		6	Serum	37.28	+	32.06	37.99	+
		6	Urine	26.93	+	29.28	25.90	+
128	M	2	Serum	29.58	+	29.62	28.76	+

Table 17: (continuation)

Patient ID	Gender	Days after Onset of Symptoms	Specimen Type	RealStar® Zika Virus RT-PCR Kit U.S.			Real-time RT-PCR described by Lanciotti et al.	
				Ct (FAM™)	Call	Ct (VIC™)	Ct (FAM™)	Call
129	F	3	Serum	34.26	+	30.16	33.89	+
		3	Urine	37.72	+	31.16	-	-
130	M	3	Urine	35.43	+	30.31	35.13	+
131	F	0	Serum	-	-	30.37	-	-
		0	Urine	36.28	+	29.79	36.82	+
132	F	3	Serum	30.58	+	29.49	30.10	+
133	F	4	Serum	35.20	+	32.73	35.45	+
134	F	0	Urine	-	-	29.62	-	-
135	M	4	Serum	32.37	+	31.16	32.22	+
		4	Serum	34.19	+	31.47	34.39	+
		4	Urine	33.21	+	30.41	34.16	+
136	F	0	Serum	36.58	+	32.12	37.04	+
137	F	3	Urine	32.11	+	29.89	31.41	+
138	F	2	Serum	34.84	+	30.31	34.56	+
139	M	3	Urine	33.61	+	29.59	33.88	+
140	M	2	Serum	31.01	+	30.44	30.39	+
		2	Urine	33.44	+	30.17	33.48	+
141	F	4	Urine	31.12	+	29.59	30.32	+
142	F	3	Serum	33.65	+	29.54	33.50	+
143	F	0	Serum	29.11	+	30.82	28.39	+
		0	Urine	35.88	+	29.89	37.81	+
144	F	0	Serum	31.06	+	29.53	30.12	+
145	F	4	Serum	33.29	+	32.66	33.17	+
146	F	4	Urine	26.17	+	29.81	26.00	+
147	M	1	Urine	-	-	30.58	-	-
148	M	5	Urine	31.22	+	29.70	30.27	+

Table 17: (continuation)

Patient ID	Gender	Days after Onset of Symptoms	Specimen Type	RealStar® Zika Virus RT-PCR Kit U.S.			Real-time RT-PCR described by Lanciotti et al.	
				Ct (FAM™)	Call	Ct (VIC™)	Ct (FAM™)	Call
149	F	2	Serum	30.07	+	30.23	29.81	+
150	F	0	Serum	35.36	+	30.58	35.23	+
151	F	2	Serum	28.97	+	30.06	28.29	+
152	F	5	Serum	37.16	+	30.29	-	-
		5	Urine	-	-	30.05	-	-
153	F	2	Serum	-	-	30.16	-	-

*Detection of the Internal Control in the VIC™ detection channel is not required for positive results in the FAM™ detection channel. The high Zika virus load in the sample leads to an absent Internal Control signal.

Paired urine and serum specimens were collected from 52 patients in the study and were analyzed. A patient was considered infected with Zika virus (i.e. positive infection status) if the serum and/or the urine sample were tested positive for Zika virus specific RNA with the real-time RT-PCR assay described by Lanciotti et al.. The patient's infection status was considered negative if both the serum and the urine sample were tested negative with the real-time RT-PCR assay described by Lanciotti et al..

Of the 52 paired samples analyzed 23 showed a positive result for the serum and/or urine specimen (i.e. patient infection status = positive) with the real-time RT-PCR assay described by Lanciotti et al., whereas 29 paired samples were negative for both, the serum and the urine specimen (i.e. patient infection status = negative). All 23 patients with positive infection status were tested positive also with the RealStar® Zika Virus RT-PCR Kit U.S. in the serum and/or urine sample. Of the 29 paired samples from patients with negative infection status 28 were tested negative for Zika virus specific RNA in the serum as well as in the urine specimen with the RealStar® Zika Virus RT-PCR Kit U.S.. One patient with negative infection status

was tested positive in the serum sample and negative in the urine sample with the RealStar® Zika Virus RT-PCR Kit U.S..

In conclusion the positive percent agreement of the results generated with the RealStar® Zika Virus RT-PCR Kit U.S. with the results from the real-time RT-PCR assay described by Lanciotti et al. is 100.0%. The negative percent agreement between the two assays is 96.6%. The results are summarized in Table 18:

Table 18: Result summary for patient infection status (detection of Zika virus RNA in serum and/or urine from patients with paired serum/urine specimens taken)
Total number of paired samples was 52.

Results from real-time RT-PCR assay described by Lanciotti et al.	Results from RealStar® Zika Virus RT-PCR Kit U.S.		
	Positive		Negative
23 Positive	23†		0
29 Negative	1*		28
Total (52 paired samples)	24		28
			95% CI
Positive Percent Agreement	23/23	100.0%	85.7%-100.0%
Negative Percent Agreement	28/29	96.6%	82.8%-99.4%

*This patient was positive only in the serum sample and negative in the urine sample with the RealStar® Zika Virus RT-PCR Kit U.S.

† Four of these patients were positive only in the urine sample and negative in the serum sample for both assays. Two patients were positive in the serum sample and negative in the urine sample with the assay described by Lanciotti et al., but positive in the serum and the urine sample with the RealStar® Zika Virus RT-PCR Kit U.S.. One patient was positive in the serum and in the urine sample with the assay described by Lanciotti et al., but positive only in the urine sample and negative in the serum sample with the RealStar® Zika Virus RT-PCR Kit U.S..

Of the 103 serum samples included in the comparison study 62 were tested positive for Zika virus RNA with the real-time RT-PCR assay described by Lanciotti et al., whereas 41 were tested negative. Of the 62 positive serum samples 60 were also tested positive with the RealStar® Zika Virus RT-PCR Kit U.S., whereas two were tested negative. Of the 41 serum samples tested negative for Zika virus with the real-time RT-PCR assay described by Lanciotti et al. 39 were tested negative and two positive with the RealStar® Zika Virus RT-PCR Kit U.S..
The results are summarized in Table 19:

Table 19: Result summary for the detection of Zika virus RNA in serum samples
Total number of serum samples was 103.

Results from real-time RT-PCR assay described by Lanciotti et al.	Results from RealStar® Zika Virus RT-PCR Kit U.S.		
	Positive		Negative
62 Positive	60		2
41 Negative	2		39
Total (103 samples)	62		41
			95% CI
Positive Percent Agreement	60/62	96.8%	89.0 % - 99.1%
Negative Percent Agreement	39/41	95.1%	83.9% - 98.7%

The performance observed using urine as only specimen type was lower than that observed when using paired specimens or serum only specimens. For this reason urine is not recommend as a sole specimen type at this present time.

15. Quality Control

In accordance with the altona Diagnostics GmbH DIN EN ISO 13485-certified Quality Management System, each lot of RealStar® Zika Virus RT-PCR Kit U.S. is tested against predetermined specifications to ensure consistent product quality.

16. Technical Assistance

For customer support, please contact our Technical Support:

e-mail: support@altona-diagnostics.com

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17. Trademarks and Disclaimers

RealStar® (altona Diagnostics GmbH); ABI Prism® (Applied Biosystems); LightCycler® (Roche); QIAamp® (QIAGEN); CFX96™ (BIO-RAD); Rotor-Gene® (Corbett Research); Rotor-Gene® (QIAGEN); FAM™, JOE™, ROX™, VIC™ (LifeTechnologies).

Registered names, trademarks, etc. used in this document, even if not specifically marked as such, are not to be considered unprotected by law.

The RealStar® Zika Virus RT-PCR Kit U.S. is for use only under Emergency Use Authorization (EUA) by specified laboratories and clinical laboratory personnel who have been trained on authorized instruments.

Not available in all countries.

18. Explanation of Symbols

IVD	*In vitro* diagnostic medical device
Rx only	For prescription use only
EUA	For use only under Emergency Use Authorization
REF	Product number
LOT	Batch code
CAP	Cap Color
USE	For use with
CONT	Content
NUM	Number
COMP	Component
GTIN	Global trade identification number
Σ	Contains sufficient for “n” tests/reactions (rxns)
	Temperature limit
	Version
	Use-by date
	Caution
	Note
	Consult instructions for use
	Manufacturer